activity via processes such as Ca^{2+}-dependent inactivation (CDI) is thought to be important in neuronal function. CDI of $Ca_V1.2$ L-channels relies upon proteins associated with the channel's C-terminus, such as the Ca^{2+}-sensor calmodulin. The C-terminus of the $Ca_V1.2$ α_1 subunit also interacts with the A-kinase anchoring protein AKAP79/150 (79 = human, 150 = rodent), which binds both protein kinase A (PKA) and the Ca^{2+}/calmodulin-activated phosphatase calcineurin (CaN). To study the roles of AKAP79/150-anchored CaN and PKA in CDI, we recorded pharmacologically-isolated L-type Ca^{2+} currents from rodent hippocampal neurons maintained in culture for up to 6 days. Rates of Ca^{2+}-dependent inactivation ($1/\tau$) were estimated by curve-fitting the fast component of the decay of Ca^{2+} current (10 mM) that occurred during 500-msec step depolarizations (0.067 Hz) from the holding potential (−60 mV) to 0 mV. In control neurons L-current underwent CDI at a rate of ~40 sec^{-1}. CDI was slowed to < 10 sec^{-1} by disruption of CaN anchoring or activity via (1) RNAi knock-down of AKAP150 coupled with over-expression of a mutant human AKAP lacking the CaN binding motif (AKAP79ΔPIX); (2) block of CaN-AKAP150 interaction by an anchoring competitor (VIVIT peptide); (3) the CaN inhibitor cyclosporine A; or (4) expression of AKAP150ΔPIX in transgenic mice. CDI was slowed to less than 20 sec^{-1} by perturbation of the PKA-AKAP interaction via (1) AKAP150 knock-down coupled with over-expression of anchoring protein lacking the PKA binding site (AKAP79ΔPKA); (2) block of PKA-AKAP150 interaction with an anchoring competitor (Ht31 peptide); or (3) expression of AKAP150ΔPKA in transgenic mice. The PKA inhibitor PKI slowed CDI to < 7 sec^{-1}.

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Ser1928 is Required for Regulation of Calcium-Dependent Inactivation of $Ca_V1.2$ L-Type Calcium Channels by AKAP79-Anchored PKA and Calcineurin

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Protein kinase A (PKA) and the Ca^{2+}/calmodulin-activated phosphatase calcineurin (CaN) reciprocally regulate $Ca_V1.2$ Ca^{2+} channel activity. The A-kinase anchoring protein AKAP79, associated with the C-terminus of $Ca_V1.2$ channels, scaffolds these opposing enzymes to the channel and, in neurons, thereby helps organize downstream signaling to the nucleus. Here we studied the role of phosphorylation signaling in CaN-mediated Ca^{2+}-dependent inactivation (CDI) of $Ca_V1.2$ channels, focusing on sites in the channel's C-terminus that can be phosphorylated by PKA (Ser1700, Thr1704, Ser1928). We used voltage-clamp recording of Ca^{2+} or Ba^{2+} current carried by $Ca_V1.2$ channels ($\alpha_1$1.2a, β_{2b}, and $\alpha_2\delta_{1a}$ subunits) transfected into tsA201 cells. Cells were also transfected with either wild-type or mutant AKAP79 constructs, and PKA activity was elevated with forskolin (FSK, 5 μM in whole-cell pipet). As previously reported, over-expression of an AKAP79 construct in which the CaN anchoring site was deleted (AKAP79ΔPIX) resulted in a decreased rate of CDI of FSK-enhanced Ca^{2+} current. Over-expression of the catalytically-inactive mutant CaN_{H151A} also resulted in a decreased rate of CDI for FSK-enhanced current. A combination of CaN inhibitors, cyclosporin A and FK506, reversibly reduced the rate of CDI for FSK-enhanced current. Ser-to-Ala substitution at Ser1928 (S1928A) reduced the rate of CDI for Ca^{2+} current in 0 FSK, but the combination of FSK (perhaps stimulating PKA action at sites other than S1928) and over-expression of AKAP79ΔPIX overcame the effect of S1928A and restored CDI to the control rate. Other sites of phosphorylation by PKA in the $Ca_V1.2$ channel were investigated as well: the decrease in rate of CDI observed for wild-type channels co-expressed with AKAP79ΔPIX was present in the double mutant S1700A+T1704A. Our results suggest that S1928 is a key structural determinant in CDI of $Ca_V1.2$ channels.

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Profile of L-Type Ca^{2+} Current and Na^+/Ca^{2+} Exchange Current during Cardiac Action Potential in Ventricular Myocytes

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Objective: The L-type Ca^{2+} current ($I_{Ca,L}$) and the Na^+/Ca^{2+} exchange current (I_{NCX}) are major inward currents that shape the cardiac action potential (AP). Previously the profiles of these currents during AP were determined from mathematical models based on data from voltage-clamp experiments that used Ca^{2+} buffer. In this study we aimed to obtain direct experimental measurement of these currents during cardiac AP with Ca^{2+} cycling. **Method:** A newly developed AP-clamp sequential dissection method was used to record ionic currents in guinea pig ventricular myocytes under a triad of conditions: (1) using the cell's own AP as the voltage command, (2) using internal and external solutions that mimic the cell's ionic milieu and, importantly, (3) no exogenous Ca^{2+} buffer was used. **Results**: The nifedipine-sensitive current (I_{NIFE}), which is composed of $I_{Ca,L}$ and I_{NCX}, revealed hitherto unreported features during AP with Ca^{2+} cycling in the cell. We identified two peaks in the current profile followed by a long residual current extending beyond the AP, coinciding with a residual depolarization. The second peak and the residual current become apparent only when Ca^{2+} is not buffered. Pharmacological dissection of I_{NIFE} using SEA0400 shows that $I_{Ca,L}$ is dominant during AP phase-1&2 whereas I_{NCX} contributes significantly to the inward current at phase-3&4. **Conclusion:** These data provide the first direct experimental visualization of $I_{Ca,L}$ and I_{NCX} during cardiac AP and Ca^{2+} cycle. The residual current reported here can serve as a potential substrate for afterdepolarizations when increased under pathological conditions.

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The Effect of Substrate Stiffness on Cardiomyocyte Calcium Currents

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The stiffness of myocardial tissue changes significantly during development and at birth, concurrent with significant changes in contractile and electrical maturation of cardiomyocytes. Previous studies by our group have shown that cardiomyocytes generate maximum contractile force when cultured on a substrate with a stiffness approximating native cardiac tissue. The force is correlated with the size of calcium transients, and on very stiff substrates this is due to lower amounts of stored calcium. However, the same relationship with stored calcium does not exist on softer substrates, and we therefore hypothesized that substrate stiffness also modifies calcium currents during cardiomyocyte action potentials. Neonatal rat ventricular cardiomyocytes (NRVMs) were isolated and cultured on polyacrylamide hydrogels of varying elastic modulus (1, 5, 10, 25, and 50 kPa). Using whole-cell patch clamping, action potential voltages were recorded in the presence and absence of BDM, a myosin inhibitor, calcium and potassium currents were recorded at voltages between −80 and 40 mV, and calcium current inactivation dynamics were determined. No significant difference was observed in potassium currents. However, the voltage at which a calcium current was at its maximum was shown to decrease with increasing stiffness, and the inactivation time constant was shown to be shorter on stiffer substrates. This can lead to less calcium influx in cells on softer substrates. Interestingly, the calcium current dynamics in NRVM on stiff substrates resemble those in adult rat cardiomyocytes. These results may explain functional differences in cardiomyocytes resulting from changes in the elastic modulus of the extracellular matrix, as observed during embryonic development, in ischemic regions of the heart after myocardial infarction, and during dilated cardiomyopathy.

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Evidence for Altered Communication Between the L-Type Ca^{2+} Channel and Mitochondria in a Model of Cardiomyopathy

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Progression of cardiac hypertrophy to failure and development of many cardiomyopathies involves myocyte remodeling, disorganisation of cytoskeletal proteins and reduced energy metabolism. The mechanisms by which cytoskeletal disruption leads to mitochondrial dysfunction are poorly understood.
Calcium influx through the L-type Ca^{2+} channel (LTCC) is a requirement for contraction in the heart. The LTCC can influence mitochondrial superoxide production, NADH production and metabolic activity in a calcium-dependent manner. Activation of the channel can also increase mitochondrial membrane potential (Ψ_m) in a calcium-independent manner. This response is dependent upon the cytoskeleton. We hypothesized that disruption of normal cytoskeletal architecture will result in altered communication between the LTCC and mitochondria. We investigated this hypothesis in the murine model of Duchenne Muscular Dystrophy(*mdx*). Myocytes from 8 week old *mdx* mice that exhibit disorganised cytoskeletal protein networks but not yet overt cardiomyopathy, demonstrated significantly slower LTCC inactivation rate (26.2 ± 1.8ms, n=13 vs 21.1 ± 1.3ms, n=16; $p<0.05$) and significantly greater calcium influx (681 ± 40nM, n=7 vs 432 ± 51nM, n=5). However activation of the LTCC did not increase Ψ_m (n=6) or metabolic activity (measured as formation of